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REVIEW

New developments providing mechanistic insight into the impact of the microbiota on allergic disease

Kathy D. McCoy^{*}, Yasmin Köller*Mucosal Immunology Lab, Department of Clinical Research, University of Bern, Murtenstrasse 35, 3010 Bern, Switzerland*

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Abstract The increase in allergic diseases over the past several decades is correlated with changes in the composition and diversity of the intestinal microbiota. Microbial-derived signals are critical for instructing the developing immune system and conversely, immune regulation can impact the microbiota. Perturbations in the microbiota composition may be especially important during early-life when the immune system is still developing, resulting in a critical window of opportunity for instructing the immune system. This review highlights recent studies investigating the role of the microbiome in susceptibility or development of allergic diseases with a focus on animal models that provide insight into the mechanisms and pathways involved. Identification of a causal link between reduced microbial diversity or altered microbial composition and increased susceptibility to immune-mediated diseases will hopefully pave the way for better preventive therapies.

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^{*} Corresponding author.

E-mail address: mccoy@dkf.unibe.ch (K.D. McCoy).

1. Introduction

The intestinal microbiota is the major component of the host-microbial superorganism when one considers the number of cells, genes and metabolic capacity that it contributes to the superorganism. Although the density and complexity of the microbiota are highest in the lower intestine, all mucosal surfaces, such as the skin, the oral cavity and the lung, harbor a resident microbiota. The microbiota of each body site has its own microbial profile and diversity index.

Many studies have linked disturbances (or dysbiosis) of the microbiota to the development or progression of diseases. Diseases that appear to be impacted by alterations in the microbiota or linked to dysbiosis include metabolic diseases, autoimmunity, inflammatory diseases, neurological diseases, and allergy (reviewed in [1]). Of note, studies linking the microbiome to allergic diseases have increased over the past years and new data supporting a causal link between low diversity microbiota and development of allergy is emerging. Already in the late eighties the British physician David Strachan proposed what is now referred to as the 'hygiene-hypothesis' [2]. Since then a steady increase in the occurrence of asthmatic and allergic diseases, such as food allergy, has been reported in industrialized countries [3]. Several environmental factors have been implicated, including decreased exposure to commensal microbes and infections, wide usage of antibiotics, water decontamination, a continuous cold-chain delivery and food pasteurization, all of which have become standard in developed countries.

The evidence for the dependence of disease development and changes in the microbiota is often deduced from clinical correlative studies in the human population. One approach of such studies is to monitor the intestinal microbial composition during active disease and/or during periods of remission and then evaluate whether active disease could be the cause or consequence of changes in microbial composition, or conversely, whether changes in the microbiota pave the way for disease development or relapse. Other studies analyze the composition of the intestinal microbiota following birth and throughout infancy and retrospectively drawing conclusions from allergy or asthma development during childhood. These studies have provided evidence that allergic diseases correlate with changes in the microbiota [4–8]. However, it is difficult to gain mechanistic insight into how bacteria can shape an allergic immune response from correlative studies. In this review we will summarize recent publications that analyze mechanisms and pathways that play a role in allergic or asthmatic diseases. We will describe what is known regarding development of the microbiota after birth and illustrate recent studies that have started to provide mechanistic understanding into how the microbiota impacts on development of allergic diseases. We provide evidence for a critical window of microbiota development that marks a crucial time in which we can profit from adequate microbial-mediated immune stimulation and highlight new studies investigating the role of the lung microbiome.

2. Intestinal microbiota

During the first three years of life our intestinal microbiota establishes an adult-like microbial community that we largely maintain for the rest of our lives [9,10]. After birth the

newborn intestine is an aerobic environment that allows inhabitation of facultative anaerobes like Enterobacteriaceae. Within a few days the lumen becomes anaerobic, thus allowing other obligate anaerobic species, for example Bifidobacteria, *Clostridium* spp. and Bacteroidetes, to populate the intestine. It is now accepted that this initial colonization is heavily influenced by the mode of delivery [11]. The microbiota of children born with cesarean section (resembling the skin microbiota) largely differs from children born vaginally (vaginal microbiota) [11]. Delivery via cesarean section correlates with delayed colonization with Bacteroidetes, a lower abundance of Bifidobacteria and Bacteroidetes, and an overall lower bacterial complexity even at one year of age [12]. Correlation studies suggest that cesarean-born children are more likely to develop obesity [13] inflammatory bowel disease (IBD) [14], or asthma or atopic diseases [15] (critically reviewed in [16]) before adulthood. During the first month of life breast or formula feeding also impacts composition of the human gut microbiota [17]. The introduction of solid food then leads to drastic shifts in composition and complexity of the intestinal microbiota. The presence of new substrates in the intestine, such as non-digestible carbohydrates, causes a shift of the microbial community to incorporate those species that can utilize and survive on the available energy sources. For example, relative proportions of Bifidobacteria or Enterobacteria decrease while relative proportions of some Clostridia species increase their relative abundance [18]. With these changes microbial composition and diversity become more 'adult-like', and also more stable (Fig. 1). The adult microbiota is represented by two main phyla: Firmicutes and Bacteroidetes. Firmicutes comprise mainly the genera *Clostridium*, *Faecalibacterium*, *Blautia*, *Ruminococcus*, and *Lactobacillus* while Bacteroidetes are mainly represented by *Bacteroides* and *Prevotella*. Less abundant are other phyla such as Actinobacteria (Bifidobacteria) or Proteobacteria (Enterobacteriaceae).

Although the gut microbiota is relatively stable in healthy adults, it is influenced by a variety of environmental factors, such as antibiotic use and the diet. It is probably not surprising that the food we consume and the relative amounts of fat, fiber or sugar play a profound role in shaping our microbiome and thereby influencing our immune system. The interplay between diet, microbiota, and the immune system is illustrated in Fig. 2. Dietary metabolites are small molecules that are derived from the food. They can be divided into microbiota-independent metabolites, such as aryl-hydrocarbon receptor ligands, retinoid acid, or folic acid or microbiota-dependent metabolites such as short-chain fatty acids (SCFAs), vitamin K or bile acids. The receptors for dietary or bacterial metabolites are widely expressed in our immune system and are particularly abundant on innate cells like macrophages or innate lymphoid cells. The interplay between the diet, metabolites and inflammation has been the subject of a recent comprehensive review [19] and therefore will not be discussed in detail here.

Recently, it was proposed to classify every human microbiome into one of three "enterotypes" [20] but until now no agreement on a "normal" or healthy microbiota has been made. Currently the ratio between the Firmicutes and Bacteroides abundances and the overall diversity of the microbiota seem to be most predictive of any correlation of the microbiome with a variety of diseases and is widely used as a read-out.

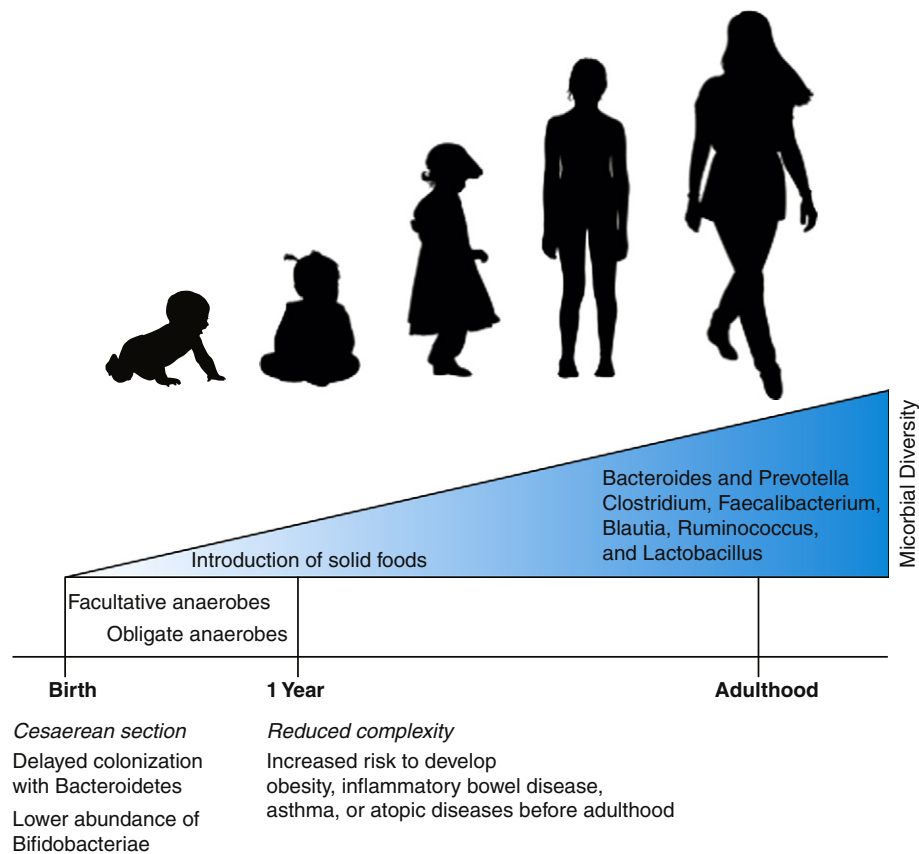


Figure 1 Microbial colonization dynamics. Diversity of the intestinal microbiota increases with age and is influenced by key life events including mode of delivery, antibiotic use, introduction of solid food, and health of the individual. Reduced microbial diversity early in life is correlated with increased risk for development of diseases.

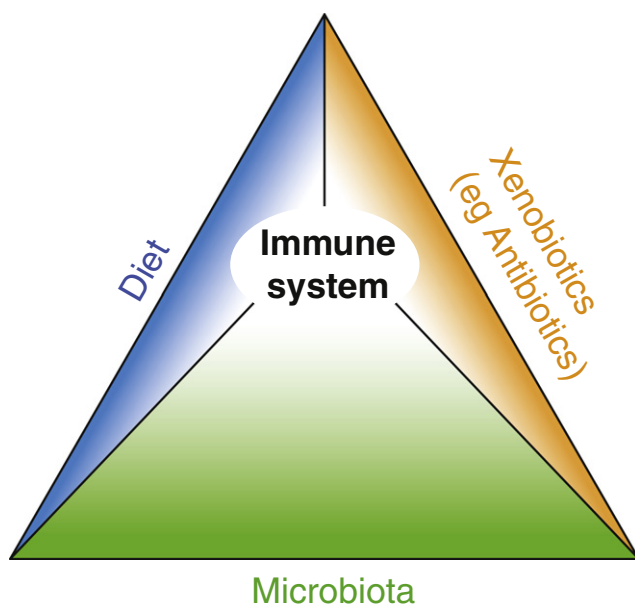


Figure 2 Interplay between diet, xenobiotics, microbiota and the immune system. The immune system is influenced by all three external factors, which also heavily impact on each other.

3. Lung microbiota

It is only recently that the existence of a lung microbiome has been appreciated and first publications have aimed to find a correlation between lung disease and the lung microbiota in humans [21] and in mice [22]. Much like the intestinal microbiota, the lung microbiota develops after birth with successive population of the tissue with bacterial species. The neonatal lung microbiome almost exclusively consists of Firmicutes and Gammaproteobacteria, while Bacteroidetes slowly expand with age until they exceed the number of Firmicutes in adults [22]. A ratio between Bacteroidetes and Firmicutes that is shifted towards Firmicutes, and thus an immature, neonatal-like microbiota, may be a predictive marker for susceptibility to asthma and development of allergies.

4. Virome

The intestinal and lung ecological habitat is not only colonized by bacteria. Notably non-bacterial organisms, particularly viruses (mainly bacteriophages but also viruses that infect host cells) and commensal fungi, are also found in this habitat and comprise the commensal virome and mycobiome, respectively. Bacteriophages are the main component of the virome.

However, in a healthy adult intestine most bacteriophages are in the lysogenic cycle [23], meaning they are integrated into the bacterial chromosome and the bacterium can continue to live and replicate normally, which is in contrast to the lytic cycle where the phage would kill the cell to generate progeny. The composition of the non-bacterial members of the microbiome is probably as diverse as the bacterial community and is likely to contribute to tissue homeostasis and disease pathogenesis. Very little of the virome sequences are defined and the lack of detailed databases and biological information makes it difficult to assign any particular biological activity to the detected sequences. It has been suggested that viruses are the most abundant entity in the biosphere (including the ocean) with an estimated number of 10^{31} [24]. A fundamental characteristic of the non-bacterial community is that the mutation rate is very high and the gene pool is large and diverse giving it a powerful potential to affect neighboring communities. Further studies in this emerging field may reveal a role for bacteriophages in shaping the gut microbiota and any potential contribution to disease [25–27].

Of particular note is the recent demonstration that an enteric virus that can infect host cells, specifically murine norovirus (MNV), can (at least partially) replace the beneficial effects of the intestinal microbiota on immune system in mice [28]. MNV is a commensal member of the mouse virome and non-pathogenic in immune sufficient animals. Administration of three strains of MNV as representatives of the mouse virome to germ-free animals could restore many features of the intestinal immune system that are attributed to bacteria, including restoration of numbers and cytokine profiles of T cells or innate lymphoid cell populations in the intestine, villus thickness and Paneth cell granularity. Future studies should provide information on the impact of the virome on susceptibility to allergic diseases.

5. The early life ‘window of opportunity’

The instability of the microbiota early in life clearly marks the most sensitive time for detrimental insults to the bacterial community. The emerging picture is that alterations that occur while the community is being established have profound and non-repairable consequences for the microbiome and subsequently for immunity. In a landmark study, Olszak et al. demonstrated that invariant natural killer T (iNKT) cells are increased in number and frequency in the colonic lamina propria and the lungs of germ-free animals [29]. A decrease in iNKT cell numbers could only be restored if mice were colonized with microbes in the first two weeks of life, but not as adults. Early life microbial exposure was important to ameliorate intestinal inflammation and development of allergic asthma following antigen challenge. Increased expression of *Cxcl16* was associated with the increase in iNKT cells and subsequent disease susceptibility, and this effect was microbial-dependent but independent of TLR adaptor protein MyD88 and likely involved epigenetic imprinting [29].

We have also described an early life “window of opportunity” whereby colonization with a diverse microbiota was required to mediate appropriate immune regulation [30]. In this study we investigated mechanisms involved in the elevated IgE in germ-free (which we also refer to as “hygiene-mediated IgE”) mice with the aim to understand

how commensal microbes can limit IgE induction and mediate immune regulation. We found that serum IgE levels started to increase in germ-free mice early in life, around 3–4 weeks of age correlating with the time of weaning. Mechanistically, B cell class switch recombination to IgE occurred in the Peyer’s patches and mesenteric lymph nodes, required IL-4 and was T cell-dependent, although MHC Class II expression on B cells was not required. In agreement with the findings of Olszak, we also found that colonization with a diverse microbiota during adulthood was not sufficient to mediate immune regulation and limit IgE induction whereas colonization of neonates was protective. Furthermore, we found that colonization with one (*Escherichia coli*) or two (*Parabacteroides distasonis* and *Lactobacillus murinus*) bacterial species, even early in life, was insufficient to limit IgE induction. In contrast, gnotobiotic mice needed to develop a diverse microbiota early in life, within this ‘window of opportunity’ in order for microbial-derived signals to mediate life-long immune regulation. In addition to limiting hygiene-mediated IgE, diverse colonization was also required to decrease susceptibility to oral antigen-induced systemic anaphylaxis [30].

Maturation of the immune system that occurs pre- and post-natally is a very important step in life and it necessitates adaptation to the environment with the possibility to respond to and tolerate surrounding antigens and toxins. These studies support the concept that an early microbial education of the immune system is required to establish a baseline immune regulation for life. Perturbations of microbial communities early in life are therefore likely to impact development of allergic disease. In support of this, administration of vancomycin, but not streptomycin, severely decreased diversity of intestinal microbiota and administration of vancomycin in neonates, but not adults, increased the severity of inflammation in a murine model of experimentally induced allergic asthma and decreased the numbers of intestinal Treg [31].

A similar time window after birth in which immune tolerance or responsiveness is established was also observed in the lung, where the commensal lung microbiota could prevent an exaggerated responsiveness to an aeroallergen only after it had shifted its composition to a greater abundance of Bacteroidetes [22]. Causal for the severe airway reaction in neonates was a reduced PD-L1 expression on DC, which resulted in a lack of Helios negative Treg cells indicating the potential lack of induction of adaptive Treg.

6. Impact of intestinal microbiota on allergic disease

Recent studies using animal models have provided some mechanistic details suggesting a causal link between alterations in the microbiota and disease, as suggested by the human correlative studies. Complete absence of the microbiota has a clear impact on immunity, as illustrated by studies in germ-free mice. Indeed, it has long been known that germ-free animals develop elevated levels of total serum IgE [32,33], a hallmark of allergy and atopy. Using a model of allergic airway inflammation, Herbst et al. demonstrated that germ-free mice develop exaggerated airway inflammation compared to mice colonized with a diverse microbiota [34]. Marked reduction in the intestinal microbiota through oral antibiotic

administration also led to increased serum IgE levels and exaggerated allergic inflammation [35]. Elevated IgE correlated with increased numbers of circulating basophils and microbial-derived signals were required to limit IgE induction and proliferation of bone marrow precursor populations [35]. Microbial control of basophil numbers may be of particular importance in light of a recent study demonstrating that IL-4 secreted from basophils controlled the function of lung natural helper cells and enhanced expression of CCL11, IL-5, IL-9 and IL-13 in a murine model of allergen-induced airway inflammation [36].

The complex network between dietary fiber, intestinal microbiota and an allergic lung reaction was recently illustrated by Trompette et al. [37]. Feeding mice with a diet high in fiber led to an increase in the relative abundance of Bacteroidaceae and Bifidobacteriaceae and subsequently increased systemically circulating levels of SCFA. The availability of higher systemic SCFA stimulated DC hematopoiesis in the bone marrow, which was ultimately associated with a less pronounced allergic lung reaction to house dust mite. In this allergic mouse model reduced eosinophil infiltration, a less activated phenotype of CD11b^{high} DC and an impaired Th2 response were also observed in mice administered with the SCFA propionate. Surprisingly, the protective process required the SCFA receptor GPR41 and not GPR43, which has previously been reported to be crucial in mediating protective anti-inflammatory effects in models of colitis or arthritis [38]. A critical aspect of this publication is that it describes the chain of events from dietary fiber to systemic metabolites to bone marrow hematopoiesis ultimately leading to immune modulation in the lung.

7. Microbiota effects on oral tolerance and food allergy

There are conflicting reports on the ability of germ-free mice to respond to oral tolerance induction protocols [39–41]. Nevertheless, the key role of intestinal bacteria in the induction of Treg [42–46] and their role in oral tolerance to food (reviewed in [47]) suggest that the microbiota is implicated in the susceptibility to development of food allergy [48].

Oral tolerance to ingested dietary antigens is classically mediated by sampling of luminal antigens from DCs and subsequent migration of CD103⁺ DC with priming of naïve T cells in the mesenteric lymph nodes to become Treg [47]. Recent data suggests that this pathway is supported by the presence of the commensal microbiota [49]. This study demonstrated that macrophage-derived IL-1 β is necessary for ROR γ t⁺ innate lymphoid cells to secrete GM-CSF, a colony-stimulating factor that influences the proliferation, differentiation, function and survival of myeloid cells. Type 3 innate lymphoid cells (ILC3) were shown to be the main source of GM-CSF in the intestine, which was important to prime dendritic cells and macrophages to induce Treg. In a model of oral tolerance to ovalbumin (OVA) reduced numbers of OVA-specific Treg were induced when mice were deficient in ILC-derived GM-CSF. This study elegantly demonstrates the impact of commensal microbes on the network of the innate immune system with consequences on

adaptive immunity, although this study did not directly look at effects on food allergy.

It had previously been shown that mice that were unable to signal through TLR4 were highly susceptible to induction of food allergy [50], leading to the speculation that responses to LPS derived from the commensal microbiota dampened allergic responses to food. Indeed, neonatal administration of antibiotics also heightened the food allergy responses [50]. Mechanistic insight into the impact of intestinal microbiota on sensitization to food antigens has recently been provided by Stefka et al. [51]. The allergy-protective effects of the microbiota were found to be derived from *Clostridia* spp. present in the microbiota. Gnotobiotic mice that were colonized with a microbiota containing *Clostridia*, but not germ-free or *Bacteroides uniformis* monoassociated mice induced an antibacterial response that was characterized by an increase in IL-22 production from innate lymphoid cells and a higher production of antimicrobial peptides such as RegIII β and RegIII γ . *Clostridia*-induced induction of IL-22 within the intestinal mucosa led to reduced intestinal antigen uptake after peanut sensitization and as a consequence, reduced concentrations of food allergens in the systemic circulation, a strong reduction in total and peanut-specific IgE levels, and a less severe hypothermic response. In addition, the pool of Treg cells was increased and intestinal IgA levels were higher in mice with the *Clostridia*-containing microbiota, but this adaptive response was independent of IL-22 induction. Instead, it seems likely that bacterial metabolites like SCFAs that are known to be produced by *Clostridia* spp. and to expand the colonic Treg pool [52–54] mediate the effect on the adaptive immune system.

While this study illustrates the profound effect of intestinal microbes on susceptibility to food allergy, induction of immune regulation can also impact the microbiota. Mice that are highly susceptible to food allergy due to a gain-of-function mutation in the IL-4 receptor alpha chain (IL4ra709 mice) displayed altered intestinal microbial profiles following oral allergic sensitization compared to sensitized control animals [55]. Of note, transfer of allergen-specific Treg not only suppressed allergic sensitization and allergen induced anaphylaxis, but also protected from development of microbial dysbiosis [55], providing evidence for host-microbial cross-talk in allergic sensitization.

8. Concluding remarks

Colonization with a diverse microbiota early in life appears to be critical for instructing regulation on the developing immune system. Failure to achieve a certain level of diversity, or absence of key microbial constituents, during a critical window of opportunity early in life leads to a dysregulated immune system similar to what is found in germ-free mice. Further understanding of the mechanisms by which microbes educate the immune system during early life and how this impacts on development of allergic disease will be key for rationale design of microbiota-based therapies for preventive treatment regimes.

Conflict of interest statement

The author(s) declare that there are no conflicts of interest.

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